Remarks

In view of the following remarks, Applicant respectfully requests reconsideration of the claims pending in the instant application. Claims 29-61 are pending. Claims 29-61 stand rejected. Below, Applicant addresses each of the objections and rejections in the order in which they appear in the Office Action mailed February, 7, 2003.

The Examiner objects to the disclosure because the Examiner believes that the specification contains an incorrect description of murine LERK-6. The basis for this conclusion stems from an error in the 1.131 Declaration of Cerretti. Specifically, the Cerretti Declaration, at page 6 first full paragraph, indicates that the "DNA encoding the first 5 amino acids shown in Appendix E is derived from the sequencing vector, as indicated by the mark between the fifth amino acid (Arg) and the sixth amino acid (Ala)." In fact, the Cerretti Declaration misstates the DNA derived from the sequencing vector. This sentence of the Cerretti Declaration should read "DNA encoding the first 3 amino acids shown in Appendix E is derived from the sequencing vector, as indicated by the mark between the ninth nucleotide (G of CGG, which encodes Arg) and the tenth nucleotide (G of GCC which encodes Ala)". The first nine nucleotides encode the first three amino acids indicated in the first amino acid sequence of Appendix E. Thus the first THREE amino acids, not the first five amino acids, are part of the sequencing vector. It should be noted that the polypeptide of SEQ ID NO:2 is 184 amino acids and with ALAARG included in the polypeptide sequence the polypeptide is 184 amino acids. So, the first 7 amino acids describing the LERK-6 shown in Appendix E are AlaArgAlaAsnAlaAspArg. These amino acids correspond to the same first 7 amino acids of SEQ ID NO:2. In order to correct the record, Dr. Cerretti has corrected and re-executed his 1.131 Declaration, a copy of which accompanies this paper. The sequence information in the 1.131 Declaration and SEQ ID NO:1 and SEQ ID NO:2 of the specification do not contradict each other and Applicant respectfully requests that this objection be withdrawn.

The Examiner rejects claims 58-60 under 35 U.S.C. 112, first paragraph, because the Examiner believes that the disclosure is not enabling for claims that are drawn to polypeptides that are at least 80% identical to the polypeptide of SEQ ID NO:2. In order to more specifically describe the invention of these claims and to overcome the rejection, Applicant amends claims 58-60 to specify that the claimed DNAs encode polypeptides that bind hek/elk. In view of this amendment, Applicant believes that this rejection is overcome and respectfully requests that this rejection be withdrawn.

The Examiner further rejects claims 29-57 and 61 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner asserts that claims 29-57 are generally drawn to an isolated molecule encoding LERK-6 polypeptide the binds hek/elk, wherein said polypeptide comprises amino acids of SEQ ID NO:2 and recombinant

phage γgt10 vector clone. The basis for the indefiniteness rejection appears to be that the Examiner believes SEQ ID NO:2 describes a chimeric protein, not murine LERK-6 polypeptide. Applicant respectfully submits that any belief that SEQ ID NO:2 is a chimeric protein is removed by the corrected 1.131 Cerretti Declaration and respectfully requests that this rejection be withdrawn.

The Examiner rejects claim 61 as being indefinite because it is drawn to various DNAs that hybridize to SEQ ID NO:1 under highly stringent conditions and the Examiner asserts that without specifically reciting conditions the claim fails to define the metes and bounds of the DNAs. Notwithstanding Applicant's view that hybridization conditions, whether it be mild, moderate, high, severe, etc, are generally viewed within the art to be within certain definite temperature and solvent ranges, in order to overcome this rejection Applicant amends claim 61 to specifically recite hybridization conditions. In view of this amendment, Applicant respectfully requests that this rejection be withdrawn.

Finally, the Examiner rejects claims 29-52 and 58-61 under 35 U.S.C. 102(e) as being anticipated by Flanagan et al. U.S. Patent No. 5,795,734 ('734 patent). The Examiner asserts that Flanagan's SEQ ID NO:2 is 100% identical to SEQ ID NO:2 of the instant claims. Flanagan's '734 patent presents claims to DNA encoding SEQ ID NO:2 and Applicant presents claims to DNA encoding SEQ ID NO:2. Since Applicant's claims have been pending since 1994 and Applicant has made a prima facie showing that Applicant is entitled to judgment relative to patentee (see Cerretti 1.131 Declaration), Applicant requests that the Examiner indicate the present claims are allowable. Pursuant to 37 C.F.R. 607, Applicant may seek to request an interference with the '734 patent.

In view of the foregoing remarks and amendment Applicant respectfully submits that the claims in this application are in condition for allowance and a notice to the effect is respectfully requested.

Respectfully submitted,

Janis C. Henry
Attorney for Applicant
Reg. No. 34,347

Immunex Corporation
Law Department
51 University Street
Seattle, WA 98101
Telephone: (206) 587-0430

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date indicated below.

Date: Aug. 7, 2003

Signed: Nanci M. Kertson
Nanci M. Kertson

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS P. CERRETTI

Appln. No. 08/538,709 Group Art Unit: 1647

Filed: October 3, 1995 Examiner: Draper, G.

For: DNA ENCODING CYTOKINE DESIGNATED LERK-6

Corrected

DECLARATION UNDER 37 C.F.R. § 1.131

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

I, DOUGLAS P. CERRETTI do hereby declare and state:

I am the inventor of the invention disclosed and claimed in the above-mentioned application.

I am familiar with U.S. Patent 5,795,734, issued to Flanagan on August 18, 1998 (hereinafter the "Flanagan Patent"), from U.S. Patent Application Serial No. 08/455,001, filed May 31, 1995 (hereinafter the "Flanagan Application"), which I understand is a Continuation-In-Part of Serial No. 08/393,461, filed February 27, 1995 (hereinafter the "Flanagan Parent Application"), which is a Continuation-In-Part of Serial No. 08/308,814, filed September 19, 1994 (hereinafter the "Flanagan Grandparent Application").

The Flanagan Patent discloses DNA encoding Elf-1, a mouse polypeptide which is now known in the art as mouse LERK-6.

In order to demonstrate and establish, *inter alia*, that I conceived and reduced to practice a DNA molecule encoding a polypeptide having mouse LERK-6 sequences in the United States by at least September 15, 1994, i.e., prior to the

September 19, 1994, filing date of the Flanagan Grandparent Application, copies of the following laboratory notebook pages and related materials, identified in detail below, are provided herewith in Appendices A-G.

I declare and state that although all of the dates from the laboratory notebook pages and related materials have been removed, all of the dates are prior to at least September 15, 1994.

Appendix A contains pages from Nicole Nelson's Laboratory Notebook No. 4266 (Bates Nos. 0001-0007). At the time, Nicole Nelson was a Research Assistant who worked under my direction and supervision at Immunex Corporation.

Appendix B contains a copy of U.S. Patent 5,516,658 (Bates Nos. 0008-0026).

Appendix C contains oligonucleotide request forms prepared by Carl Kozlosky (Bates Nos. 0027-0030). At the time, Carl Kozlosky was a Research Associate who worked under my direction and supervision at Immunex Corporation.

Appendix D contains pages from Carl Kozlosky's Laboratory Notebook No. 3388 (Bates Nos. 0031-0037).

Appendix E contains various computer printouts of LERK sequences that I personally generated (Bates Nos. 0038-0049).

Appendix F contains the minutes of an internal HEK/ELK meeting at Immunex Corporation that was chaired by Barry Davison in my absence (Bates Nos. 0050-0051). Barry Davison, who prepared the minutes, was the Director of the Transgenics Department at Immunex Corporation at the time.

Appendix G contains a copy of American Type Culture Collection Form BP4/9 for ATCC deposit No. 75829 (Bates No. 0052).

Prior to September 15, 1994, and as described in Example 1 of the present application, a DNA molecule encoding mouse LERK-6 was isolated under my direction and supervision. The specific experiments detailed below were carried out at my behest and command and under my direction and supervision by scientists at Immunex Corporation.

More specifically, prior to September 15, 1994, and as described at page 23, lines 5-7 of the present application, a commercially available 11.5 day murine embryonic cDNA library was obtained by Nicole Nelson from Clonetech Laboratories, Inc., Palo Alto, California (**Appendix A**, Bates No. 0003).

Next, prior to September 15, 1994, and as described on page 23, lines 7-8 of the present application, the library was plated by Nicole Nelson according to the procedures detailed in the manual provided by Clonetech (**Appendix A**, Bates No. 0004). The initial purpose of these efforts was to clone the cDNA for mouse LERK-3 (referred to as A2) and mouse LERK-4 (referred to as C6). However, instead a new mouse LERK molecule was discovered, i.e., mouse LERK-6.

Prior to September 15, 1994, and as described on page 23, lines 8-30 of the present application, probes, referred to as A2 (LERK-3) and C6 (LERK-4), were generated using standard techniques. Generally, polymerase chain reaction (PCR) (Mullis et al, *Meth. Enzymol.*, 155:335-350 (1987)) amplifications were performed by Carl Kozlosky (**Appendix D**, Bates Nos. 0036-0037) using two sets of primers. The first set of primers,

GATATTTACT GCCCGCACTA CAACAGCT	SEQ ID NO:3
AGAGAAGGCG CTGTAGCGCT GGAAC	SEQ ID NO:4

was used to generate amplified double stranded DNA fragments from the DNA of LERK-3 (LERK-3, also known as hek-ligand, is the subject of U.S. Patent 5,516,658 **(Appendix B,** Bates Nos. 0008-0026) which issued from U.S. Patent Application Serial No. 08/240,124, filed May 9, 1994, and which claims benefit of Serial Nos. 08/109,745, 08/114,426 and 08/161,132). The probe from LERK-3 comprised nucleotides 260 through 481 of the SEQ ID NO:1 of U.S. Patent 5,516,658. The second set of primers,

ACGTAGTCTA CTGGAACTCC AGTAACCCCA G SEQ ID NO:5

AGCCTCAAGC ACTGGCCAGA ACTCTCTCTG GAGT SEQ ID NO:6

was used to generate amplified double stranded DNA fragments from the DNA of LERK-4 (LERK-4 also is the subject of U.S. Patent 5,516,658). The probe from LERK-3 comprised nucleotides 110 through 467 of the SEQ ID NO:3 of U.S. Patent 5,516,658.

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:3, i.e., oligo #12334 (also referred to as A2rib5.28) **(Appendix C,** Bates No. 0027), and the core facility actually synthesized such prior to September 15, 1994, as shown in **(Appendix C,** Bates No. 0027).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:4, i.e., oligo #12333 (also referred to as A2T7.49) **(Appendix C,** Bates No. 0028), and the core facility actually synthesized such prior to September 15, 1994, as shown in **(Appendix C,** Bates No. 0028).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:5, i.e., oligo #12312

(also referred to as C6RIBO5.31) (**Appendix C**, Bates No. 0029), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0029).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:6, i.e., oligo #12316 (also referred to as C6T7.54) (**Appendix C**, Bates No. 0030), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0030).

Oligonucleotides #12333 and 12316 also included nucleotides encoding the T7 polymerase promoter.

Prior to September 15, 1994, Carl Kozlosky identified the location of oligonucleotides #12334 (A2rib5.28) and #12333 (A2T7.49) in the LERK-3 DNA sequence, as shown in **Appendix D**, Bates Nos. 0032-0033); as well as the location of oligonucleotides #12312 (C6RIBO5.31) and 12316 (C6T7.54) in the LERK-4 DNA sequence, as shown **Appendix D**, Bates Nos. 0034-0035).

Thereafter, and prior to September 15, 1994, and as described on page 23, lines 30-35 of the present application, the PCR fragments (A2/LERK-3 and C6/LERK-4) were radiolabelled by Nicole Nelson with ^{32}P (**Appendix A**, Bates No. 0005), and used by Fred Fletcher as probes to screen the murine embryonic cDNA library prepared by Nicole Nelson by conventional procedures, and hybridizing clones were identified. Fred Fletcher was a Staff Scientist at Immunex Corporation at the time who worked on the LERK project under my direction and supervision. The hybridizing conditions consisted of 42°C and 50% Starks washed to 0.1X SSC at 63°C (**Appendix A**, Bates Nos. 0005-0006). In this manner, clone #13 was identified by Fred Fletcher on an X-ray

film, a copy of which was placed in Nicole Nelson's Laboratory Notebook (**Appendix A**, Bates No. 0007).

Then, and prior to September 15, 1994, and as described on page 23, line 35 and page 24, line 4, of the present application, the nucleotide sequence of the cDNA insert of clone #13 (λ13), isolated from the murine embryonic cDNA library, i.e., mouse LERK-6 (mLERK6), was determined at my request by the core facility at Immunex Corporation, and the results were entered into a computer database, a printout of which, which was generated prior to September 15, 1994, is shown in **Appendix E**, Bates Nos. 0038-0039. DNA encoding the first 3 ~~5~~ amino acids shown in **Appendix E** is derived from the sequencing vector, as indicated by the mark between the ~~fifth amino acid (Arg)~~ ninth nucleotide G of CGG and the ~~sixth amino acid (Ala)~~ tenth nucleotide G of GCC. Also, the initiation codon Met is not shown in **Appendix E**. Thus, a substantially complete cDNA sequence of the coding region of the clone λ13 cDNA, and the amino acid sequence encoded thereby were determined, and are presented in SEQ ID NO:1 and SEQ ID NO:2, respectively of the present application. The open-reading frame within this sequence in **Appendix E** (and within SEQ ID NO:1) encodes a protein of 184 amino acids beginning with the ~~second~~ first Ala.

opc 6/7/2003

BBL 6/7/2003

Prior to September 15, 1994, I carried out a comparison of the amino acid sequences of mouse LERK-6 v. human LERK-3 (also referred to as A2) (**Appendix E**, Bates No. 0040); mouse LERK-6 v. human LERK-4 (also referred to as C6) (**Appendix E**, Bates No. 0041); mouse LERK-6 v. human LERK-2 (also referred to as ELKL) (**Appendix E**, Bates No. 0042); mouse LERK-6 v. human LERK-5 (**Appendix E**, Bates No. 0043); mouse LERK-6 v. human LERK-1 (also referred to as B61) (**Appendix E**, Bates No. 0044); mouse LERK-6 v. mouse LERK-4 (also referred to as MC6) (**Appendix E**, Bates

No. 0045); as well as the DNA sequences for mouse LERK-6 v. human LERK-3 (A2) (**Appendix E**, Bates Nos. 0046-0047),); mouse LERK-6 v. mouse LERK-4 (MC6) (**Appendix E**, Bates No. 0048); and mouse LERK-6 v. human LERK-5 (**Appendix E**, Bates No. 0049). These comparisons showed many conserved amino acids and nucleotides amongst the family, and clearly showed that LERK-6 was a member of the LERK family of proteins, and thus would bind to hek/elk.

Prior to September 15, 1994, the results of the above-discussed experiments were presented by Nicole Nelson at an internal HEK/ELK meeting at Immunex Corporation. This meeting was chaired by Barry Davison in my absence, who prepared the meeting minutes, a copy of which is shown in **Appendix F** Bates No. 0050-0051.

Next, as described on page 24, lines 14-17 of the present specification, on July 15, 1994, a cell lysate containing clone λ13 DNA (the LERK-6 cDNA in λgt10) was deposited with the American Type Culture Collection, Rockville, MD, USA, and assigned accession number ATCC 75829. A copy of the deposit receipt is shown in **Appendix G**, Bates No. 0052.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

as corrected pg 6

Date: 2/20/01
8/7/2003

Name: Douglas P. Cerretti
DOUGLAS P. CERRETTI

Douglas P. Cerretti

NOTEBOOK NO. 42616

ISSUED TO Nicole Nelson

ON _____ 19____

DEPARTMENT Nel Sci.

RETURNED _____ 19____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 4216 Date form completed:

Form Completed by: Neil Nelson

MOLECULE(S): c MGF

[redacted]

HEK

Alk

LERK 4

LERK 3

PROJECT(S):

0002

Page _____ of _____

Product Analysis Certificate

PRODUCT: Mouse Embryo 5'-STRETCH cDNA Library

CAT. #: ML1027a

LOT #: 1211

STORAGE CONDITIONS:
SHORT-TERM STORAGE (< 6 MONTHS)
4°C

LONG-TERM STORAGE (> 6 MONTHS)
–70°C

SHELF LIFE:
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS:
Dry Ice (–70°C)

PACKAGE CONTENTS:
- 0.2 ml library lysate in 1X Lambda Dilution B
  and 7% DMSO
- 0.5 ml host strain
- Lambda Library Protocol Handbook (PT101

TITER: $\geq 10^8$ pfu/ml

CLONING VECTOR: λgt10

CLONING SITE: *Eco*R I

PRIMING METHOD: oligo(dT)-primed

HOST STRAIN: C600 *Hfl*

mRNA SOURCE:
whole embryo (not including placenta extraembryonic membranes) from a cross betw ICR outbred females and outbred Swiss Webs males, 11.5 days post-coitus (noon on the day vaginal plug is 0.5 day post-coitus)

NOTE: No further information on the mRNA sour was made available to CLONTECH.

QUALITY CONTROL DATA

SELECTION CRITERIA: Clear plaques from turbid plaques (nonrecombinan or parental)

ESTIMATED % OF CLEAR PLAQUES: 86%
(when plated on C600 before amplifying in C600*Hfl*

NUMBER OF INDEPENDENT CLONES: 1.7×10^6

AVERAGE INSERT SIZE: 1.5 kb

INSERT SIZE RANGE: 0.8–4.0 kb

AMPLIFICATION: This library was amplified once in C600 *Hfl*.

APPROVED BY:

(PA93650-1)

0003

FOR RESEARCH USE ONLY

Project No.
cDNA for 1A1C6 Book No. 83

Page N —— Titer Library

0λ	—	
2λ	307	1.5×10^7
5λ	159	4.0
10λ	1365	3.4

} 4×10^7 pfu/λ

2λ library → 1ml SM → 2λ : 1ml SM
1:500 1:250000

Plate the cells at 5×10^4

5×10^4 pfu/plate / 4×10^7 = .00125 λ

2λ library → 1ml SM → 10:990 SM
8×10^4 8×10^3 pfu/λ

5×10^4 / 8×10^3 = 3.75 λ/plate need 7.5λ

Make a fresh dilution and plate at 3.75λ/plate (2) plated by 10AM

The plates did not grow in 8 hours & left and returned 11PM. They had grown to full size & the concentration was ~10 fold low.

0004

To Page N 8

Project No.________

TITLE 5' Stretch DNA library Murine Book No.________

Fr m Page No. 83 Probes made for Fred Fletcher to use in screening

```
            2x purified on Sephadex 2 G50 columns
PROGRAM #=  14                                              01:44
REGION A:    LL-UL=   5-1700  LCR=   0  BKG=    .00  % 2 SIGMA=   .0
REGION B:    LL-UL=  50-1700  LCR=   0  BKG=    .00  % 2 SIGMA=   .0
REGION C:    LL-UL=    0- 0   LCR=   0  BKG=    .00  % 2 SIGMA=   .0
TIME=   1.00  K= 1.000 QIP=SIS

P#  S#   TIME  CPMA/K %DEV  CPMB/K %DEV  CPMC/K %DEV  SIE    SIS FLAGS  MIN
WARNING:  NOT NORMALIZED
15   1   1.00 481546.  .29 9757.00 2.02   .00  .00 .000 41.366 ~180λ   1 A2
15   2   1.00 666133.  .25 22258.0 1.34   .00  .00 .000 45.390 ~110λ   3 C6
PROGRAM #=  13                                              23:32
REGION A:    LL-UL=   5-1700  LCR=   0  BKG=    .00  % 2 SIGMA=   .0
REGION B:    LL-UL=  50-1700  LCR=   0  BKG=    .00  % 2 SIGMA=   .0
REGION C:    LL-UL=    0- 0   LCR=   0  BKG=    .00  % 2 SIGMA=   .0
TIME=   1.00  K= 1.000 QIP=SIS

P#  S#   TIME  CPMA/K %DEV  CPMB/K %DEV  CPMC/K %DEV  SIE    SIS FLAGS  MIN
WARNING:  NOT NORMALIZED
13   1   1.00 389178.  .32 6839.00 2.42   .00  .00 .000 37.510 ~120λ   1 A2
13   2   1.00 293853.  .37 4696.00 2.92   .00  .00 .000 35.396 ~120λ   3 C6
A2 4.7x10^8 total cts                 C6 3.5x10^8 total cts
```

a Murine cDNA library for HEK C6, & A2

These probes worked well on the positive controls included in the hybridization. See Fred Fletcher for films

0005

To Page N 85

Witnessed & Understood by me, [signature] | Date | Invented by | Date | Recorded by [signature]

1.94

mouse embryo
cDNA library
probed w/ A2, C6
and GSK-β.

probed 42°C ON starks

washed to 1XSSC, 63°

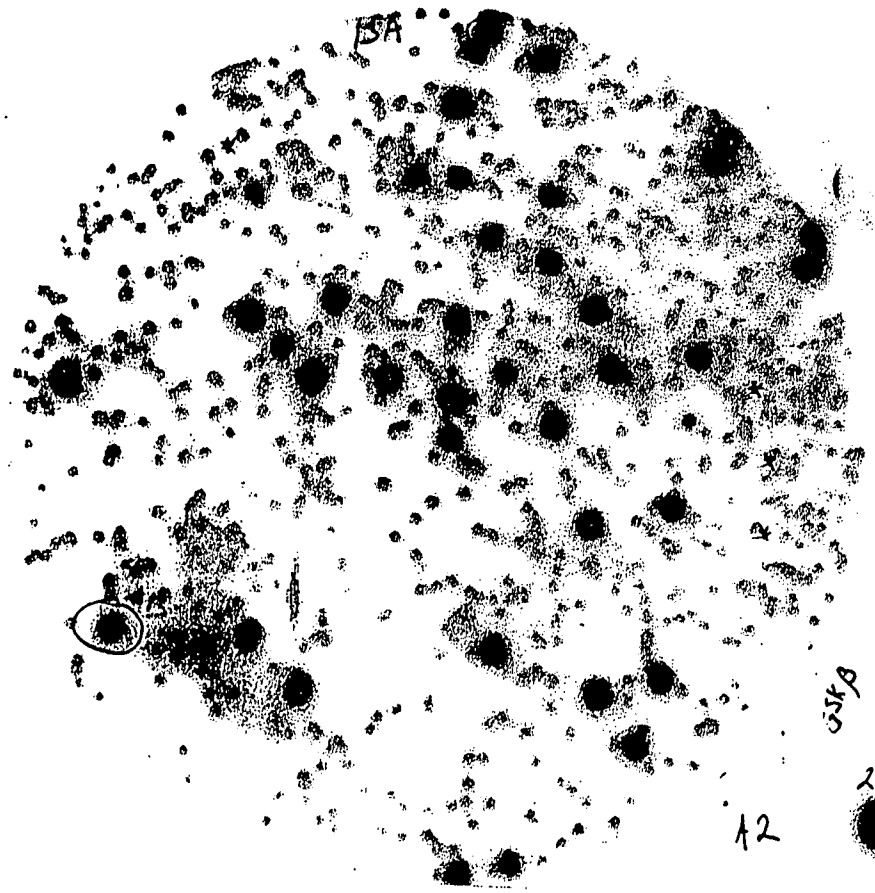
1° films
from Fred Fletcher
I plated this library before xmas 93
but recieved these films

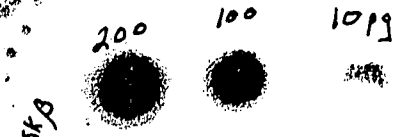
0006

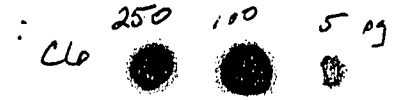
Mu. gDNA 2°

Prehyb 42° >2hrs
Hyb 42° o/n 50% STARKS [illegible] 12aCG [illegible]
[illegible] GSKβ riboprobe
Wash RT 6X SSC .1% SDS
63° 1X SSC " 60'
65° 0.1X SSC " 30'

2xp 3 days 4266/89







0007

Oligo NAME: A2RIB5.28

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

oligo number: 12334

Date Synthesized:

DNA Sequence (5'-3'): 5'-GAT ATT TAC TGC CCG CAC TAC AAC AGC T-3'

(28 bases; 8A's 4G's 9C's 7T's)

PURIFICATION: PHENOL OPC

COMMENTS:
A2 5 PRIME PCR OLIGO FOR MAKING A T7 RIBOPROBE.

R7043

A2rib5.28

1 GATATTTACT GCCCGCACTA CAACAGCT

Column 2

9:44:32A

Run ID :
Cycle : 40PLUS CYC
End Proc: End CE (DMT = On)
Sequence: 12334

Total bases = 28
A= 8, G= 4, C= 9, T= 7, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 8489.6

5'> GAT ATT TAC TGC CCG CAC TAC AAC AGC T <3'

Purification: OPC
Amount of crude: all
O.D.260: .6060
dilution factor: 1:500
concentration:
yield: 10.09 µg/µl
100.9 µg

gel on back

Oligo NAME: A2T7.49 Oligo number: 12333

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA [illegible]
GAC TCA CTA TAG AGA
GAA GGC GCT GTA CGG
CTG GAA C-3'

(49 bases)

16A's 14G's
10C's 9T's

PURIFICATION: ~~PHENOL~~ OPC

COMMENTS:
3 PRIME A2 OLIGO TO PCR A T7 RIBOPROBE. THIS OLIGO IS ANTISENSE AND CONTAINS THE T7 PROMOTER.

A2t7.49

R7044

1 TGCGAATAAT ACGACTCACT ATAGAGAAGA|GGCGCTGTAG CGCTGGAAC

Column 1

9:44:31A

Run ID :
Cycle : 40PLUS CYC
End Proc: End CE (DMT = On)
Sequence: 12333

Total bases = 49
A= 16, G= 14, C= 10, T= 9, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 15174.8

5'> TGC GAA TAA TAC GAC TCA CTA TAG AGA GAA GGC GCT GTA

GCG CTG GAA C <3'

Purification: OPC
Amount of crude: all
O.D.260: .276
dilution factor: 1:500
concentration: 4.60 µg/λ
yield: 460 µg

gel on 12334

Oligo NAME: C6RIBO5.31

Oligo number: 12312

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-ACG TAG TCT ACT
GGA ACT CCA GTA ACC
CCA G-3 '

(31 bases)

9A's 6G's
10C's 6T's

PURIFICATION: ~~CHROM~~ OPC

COMMENTS:
5 PRIME PCR FOR C6 RIBO

R7023

Column 2

3:48:43P

Run ID :
Cycle : 40PLUS CYC
End Proc: End CE (DMT = On)
Sequence: 12312
Bo

Applied Biosystems G 209118

Total bases = 31
A= 9, G= 6, C= 10, T= 6, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 9444.2

5'> ACG TAG TCT ACT GGA ACT CCA GTA ACC CCA G <3'

Purification: OPC
Amount of crude: all
O.D.260: 0.382
dilution factor: 1-500
concentration: 6.36 ug/x
yield: 636 ug

gel on 12,334

0029

Oligo NAME: C6T7.54

Oligo number: 12316

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA TAA TAC
GAC TCA CTA TAG CCT
CAA GCA CTG GCC AGA
ACT CTC TGG AGT -3'

(54 bases) 16A's 11G's
15C's 12T's

PURIFICATION: ~~PHENOL~~ OPC

COMMENTS:
C6 3 PRIME FOR C6 RIBO
USE T7 POL.

Applied Biosystems T 453741

R7024

COLUMN 2 SET-UP
VERSION 2.02

USER_NAME:
CYCLES USED: 0.2UMB - 1
ENDING METHOD: Trityl ON, Auto
ENDING PROCEDURE: deproc
SEQUENCE NAME: 12316
SEQUENCE LENGTH: 54
DATE:
TIME: 17:37
COMMENT:

5'- TGC GAA TAA TAC GAC TCA CTA TAG CCT CAA GCA CTG

GCC AGA ACT CTC TGG AGT -3'

yield:

OPC
all
0.303
1:50
5.04 ug/x
504 ug

gel on
12334

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 3388 Date form completed:

Form Completed by: Carl Kozlosky

MOLECULE(S): B61, ELK, ELK-L, HEK, LeRKs 1, 2, 3, 4, 5, 7

PROJECT(S): RISOL

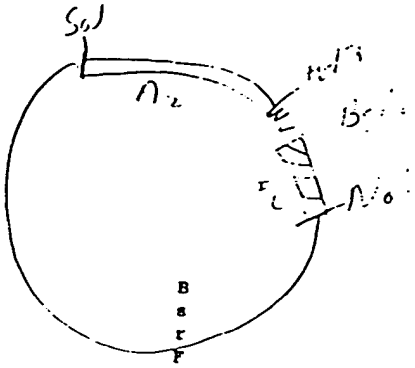
(Linear) (Six Base) MAP of: A2.Seq check: 6473 from: 1 to: 1037

HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL]

With 114 enzymes: *

T7 is 5' End
Bgl2 into Bam HI

09:13 ..



Xho21 EcoP31 BspMI1 BpmI1 BsaBI26 BsrFI1 NspBII2

1 GGATCTTGGAACGAGACGACCTGCTGGAGAAGCCGGGAGCGCGGGGCTCAGTCGGGGGGCGGCGGCGGCGGCGGCTCCGGGGATGGCGGCGGCTCCGCTG 100
CCTAGAACCTTGCTCTGCTGGACGACCTCTTCGGCCCTCGCGCCCCGAGTCAGCCCCCCGCCGCCGCCGCGCCGAGGCCCCTACCGCCGCCGAGGCGAC
AspLeuGlyThrArgArgProAlaGlyGluAlaGlySerAlaGlyLeuSerArgGlyAlaAlaAlaAlaAlaProGlyMetAlaAlaAlaProLeu –

BspI286 NspBII2 NspBII2 StyI1 DraII2 BsaBIpFan811261 HaeII2 NspBIIHh11

101 CTGCTGCTGCTGCTGCTCGTGCCCGTGCCGCTGCTGCCGCTGCTGGCCCAAGGGCCCGGAGGGGCGCTGGGAAACCGGCATGCGGTGTACTGGAACAGCT
GACGACGACGACGACGAGCACGGGCACGGCGACGACGGCGACGACCGGGTTCCCGGGCCTCCCCGCGACCCTTTGGCCGTACGCCACATGACCTTGTCGA
LeuLeuLeuLeuLeuLeuValProValProLeuLeuProLeuLeuAlaGlnGlyProGlyGlyAlaLeuGlyAsnArgHisAlaValTyrTrpAsnSerSer –

AlwNI1 BspMI1 BspMI1

A2R185.28

AvaI1 DraII2 AAvpaa11

201 CCAACCAGCACCTGCGGCGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATCTGGATATTTACTGCCCGCACTACAACAGCTCGGGGGGTGGGCCC
GGTTGGTCGTGGACGCCGCTCTCCCGATGTGGCACGTCCACTTGCACTTGCTGATAGACCTATAAATGACGGGCGTGATGTTGTCGAGCCCCCACCCGGG
AsnGlnHisLeuArgArgGluGlyTyrThrValGlnValAsnValAsnAspTyrLeuAspIleTyrCysProHisTyrAsnSerSerGlyValGlyPro –

BspPBBlsPSXa2rsmn8Fsaa261111 DraII2 BsaBIpFan811261 BsaAI1 BglI1 BspMI1

301 CGGGGCGGGACCGGGGCCCGGAGGCGGGGCAGAGCAGTACGTGCTGTACATGGTGAGCCGCAACGGCTACCGCACCTGCAACGCCAGCCAGGGCTTCAAG
GCCCCGCCCTGGCCCCGGGCCTCCGCCCCGTCTCGTCATGCACGACATGTACCACTCGGCGTTGCCGATGGCGTGGACGTTGCGGTCGGTCCCGAAGTTC
GlyAlaGlyProGlyProGlyGlyGlyAlaGluGlnTyrValLeuTyrMetValSerArgAsnGlyTyrArgThrCysAsnAlaSerGlnGlyPheLys –

EcoHaeIII47321

AZT7.49

EcoHSafec32121 HaeII2 NgoMI1

401 CGCTGGGAGTGCAACCGCCCGCACGCGCCGCACAGCCCCATCAAGTTCTCGGAGAAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCACG
GCGACCCTCACGTTGGCGGGCGTGCGCGGCGTGTCGGGGTAGTTCAAGAGCCTCTTCAAGGTCGCGATGTCGCGGAAGAGAGACCCGATGCTCAAGGTGC
ArgTrpGluCysAsnArgProHisAlaProHisSerProIleLysPheSerGluLysPheGlnArgTyrSerAlaPheSerLeuGlyTyrGluPheHisAla –

EaeI1 ScaI1 BspMI1 XmnI BsrI

CCGGCCACGAGTACTACTACATCTCGAGCGGC

0032

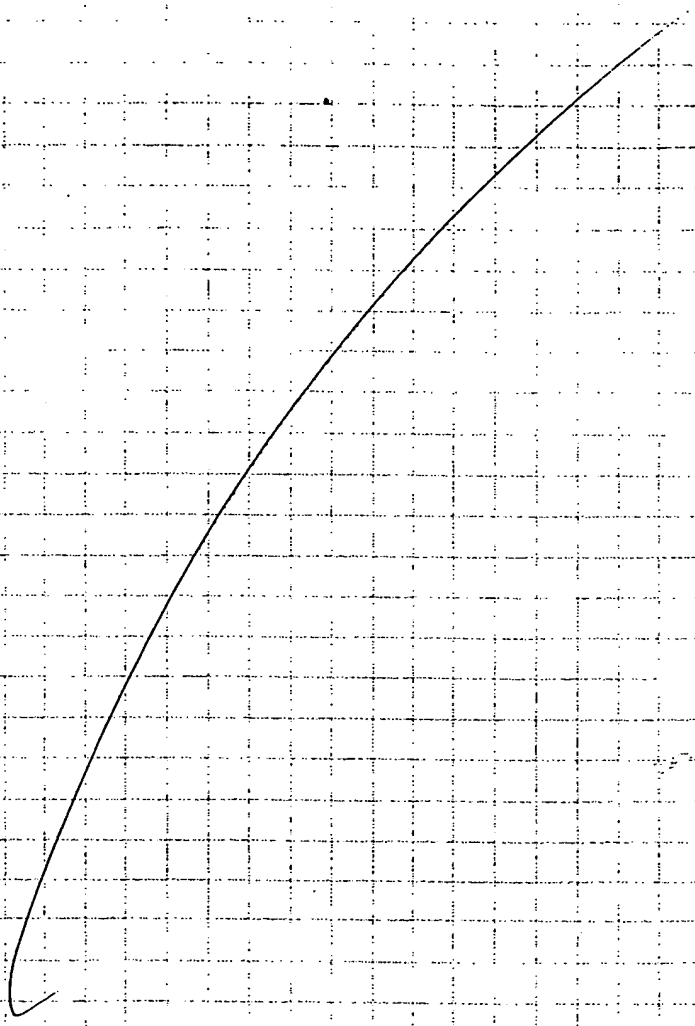
TITLE A2 Seq.

Project No.

Book No.

62

From Page No.



To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
apc		Recorded by	

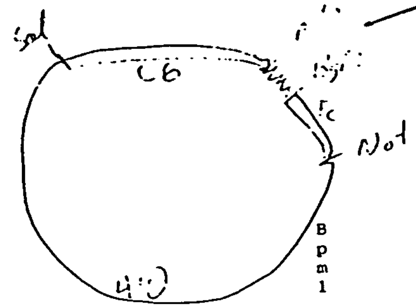
0033

(Linear) (Six Base) MAP of: C6.seq check: 9352 from: 1 to: 636

HEKL 132-11, C6-no vector
2491,T7,DPC3266,DPC3267,~~DPC3274~~,DPC3275
SR1810 KOZLOSKY
file: [BERTLESJ.HEKL]C6.SEQ

With 114 enzymes: *

12:12 ..




```
Enzymes: AvaI, BcgI, BpmI, BspB1286I

GCCAGACCAAACCGGACCTCGGGGGCGATGCGGCTGCTGCCCCTGCTGCGGACTGTCCTCTGGGCCGCGTTCCTCGGCTCCCCTCTGCGCGGGGGCTCCA
1 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 100
CGGTCTGGTTTGGCCTGGAGCCCCCGCTACGCCGACGACGGGGACGACGCCTGACAGGAGACCCGGCGCAAGGAGCCGAGGGGAGACGCGCCCCCGAGGT

a: AlaArgProAsnArgThrSerGlyAlaMetArgLeuLeuProLeuLeuArgThrValLeuTrpAlaAlaPheLeuGlySerProLeuArgGlyGlySerSer -

Enzymes: BsaI, BpmI, AccI, Esp3I, BsaHI, DsaI, Tth3I
C6RIBO.31

GCCTCCGCCACGTAGTCTACTGGAACTCCAGTAACCCCAGGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCCTCAACGATTACCTAGACATTGTCTG
101 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 200
CGGAGGCGGTGCATCAGATGACCTTGAGGTCATTGGGGTCCAACGAAGCTCCTCTGCGGCACCACCTCGACCCGGAGTTGCTAATGGATCTGTAACAGAC

a: LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys -

Enzymes: DraII, DraII, PpuMI, AvaII, BsaJI, Bsu36I, BspB1286I, PssI, DraII, DraII, Esp3I, PssI, ApaI, AvaII, BanII, Bsp1286I, BcgI, PssI, EaeI, BalI, DraII, DraII, PssI, ApaI, AvaII, BanII

CCCCCACTACGAAGGCCCAGGGCCCCCTGAGGGCCCCGAGACGTTTGCTTTGTACATGGTGGACTGGCCAGGCTATGAGTCCTGCCAGGCAGAGGGCCCC
201 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 300
GGGGGTGATGCTTCCGGGTCCCGGGGGACTCCCGGGGCTCTGCAAACGAAACATGTACCACCTGACCGGTCCGATACTCAGGACGGTCCGTCTCCCGGGG

a: ProHisTyrGluGlyProGlyProProGluGlyProGluThrPheAlaLeuTyrMetValAspTrpProGlyTyrGluSerCysGlnAlaGluGlyPro -

Enzymes: BspB1286I, BsrFI, PssI, SmaI, XmaI, Eco47III, HaeII, BspHI, BglI, BsiAI, EaeI, BalI, Eco47III, HaeII

CGGGCCTACAAGCGCTGGGTGTGCTCCCTGCCCTTTGGCCATGTTCAATTCTCAGAGAAGATTCAGCGCTTCACACCTTTCTCCCTCGGCTTTGAGTTCT
301 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 400
GCCCGGATGTTCGCGACCCACACGAGGGACGGGAAACCGGTACAAGTTAAGAGTCTCTTCTAAGTCGCGAAGTGTGGAAAGAGGGAGCCGAAACTCAAGA

a: ArgAlaTyrLysArgTrpValCysSerLeuProPheGlyHisValGlnPheSerGluLysIleGlnArgPheThrProPheSerLeuGlyPheGluPheLeu -

Enzymes: BpmI, BpmI, BanI, Bsp1286I, Tth32I, EaeI, BapI, BamlI

TACCTGGAGAGACTTACTACTACATCTCGGTGCCCACTCCAGAGAGTTCTGGCCAGTGCTTGAGGCTCCAGGTGTCTGTCTGCTGCAAGGAGAGGAAGTC
401 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 500
ATGGACCTCTCTGAATGATGATGTAGAGCCACGGGTGAGGTCTCTCAAGACCGGTCACGAACTCCGAGGTCCACAGACAGACGACGTTCCTCTCCTTCAG

a: ProGlyGluThrTyrTyrTyrIleSerValProThrProGluSerSerGlyGlnCysLeuArgLeuGlnValSerValCysCysLysGluArgLysSer -
GATATCACTCAGCATAATAAGCGT
C6T7.54

Enzymes: BstXI, BspB1286I, BpmI
A

TGAGTCAGCCCATCCTGTTGGGAGCCCTGGAGAGAGTGGCACATCAGGGTGGCGAGGGGGGGACACTCCCAGCCCCCTCTGTCTCTTGCTATTACTGCTG
501 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 600
ACTCAGTCGGGTAGGACAACCCTCGGGACCTCTCTCACCGTGTAGTCCCACCGCTCCCCCCCTGTGAGGGTCGGGGGAGACAGAGAACGATAATGACGAC
TCTAG
```

179 aa ≈ 20 Kd

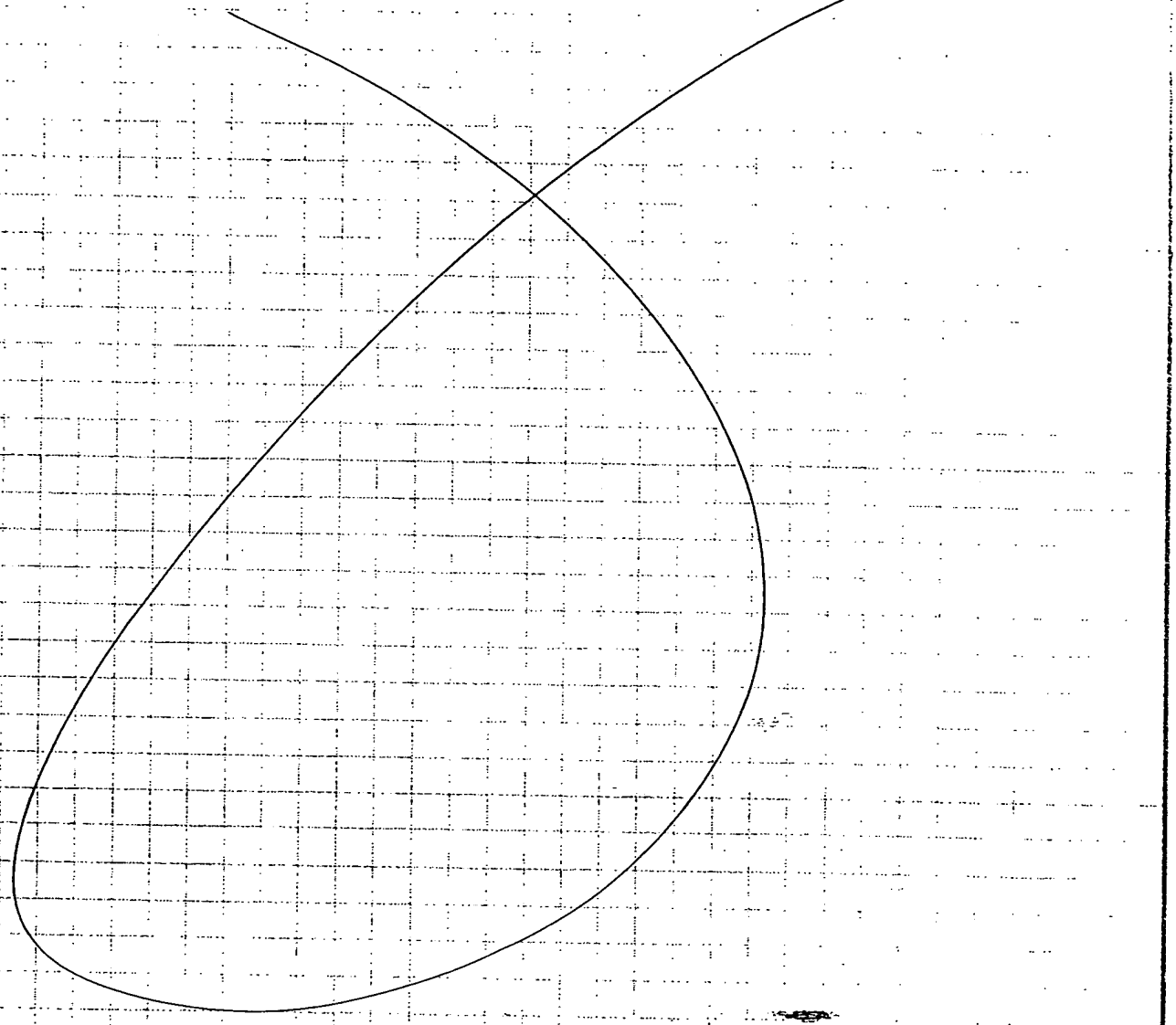
TITLE Cb Sag.

Project No._____

Book No._____

6(

From Page No.__



To Page No.____

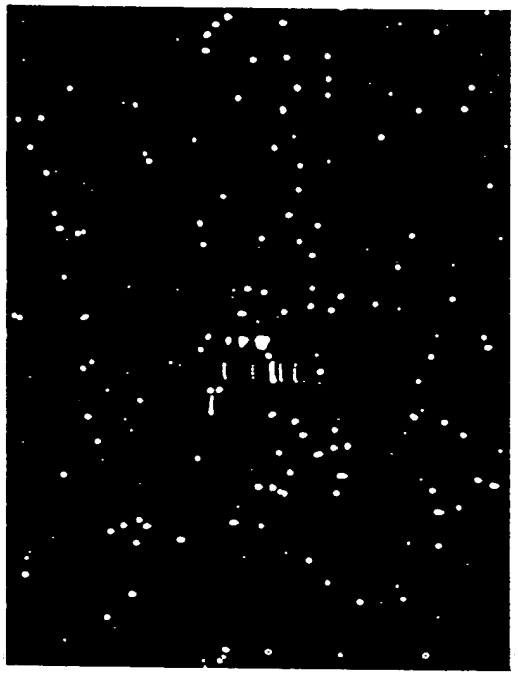
Witnessed & Understood by me,	Date	Invented by	Date
pc		Recorded by Carl Korlesky	

TITLE C6 T7 Ribo PCR

Project No.

Book No.

From Page No.



C6 Binding Region
T7 RNA Pol

To Page No.

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by	

0036

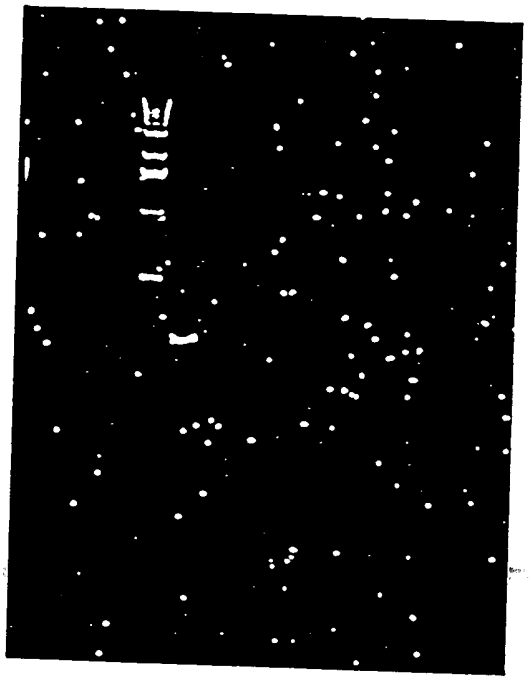
TITLE Cb 1st

Project No.

Book No.

From Page No.

< 185 ~ 1.6Kb IN HSB-2



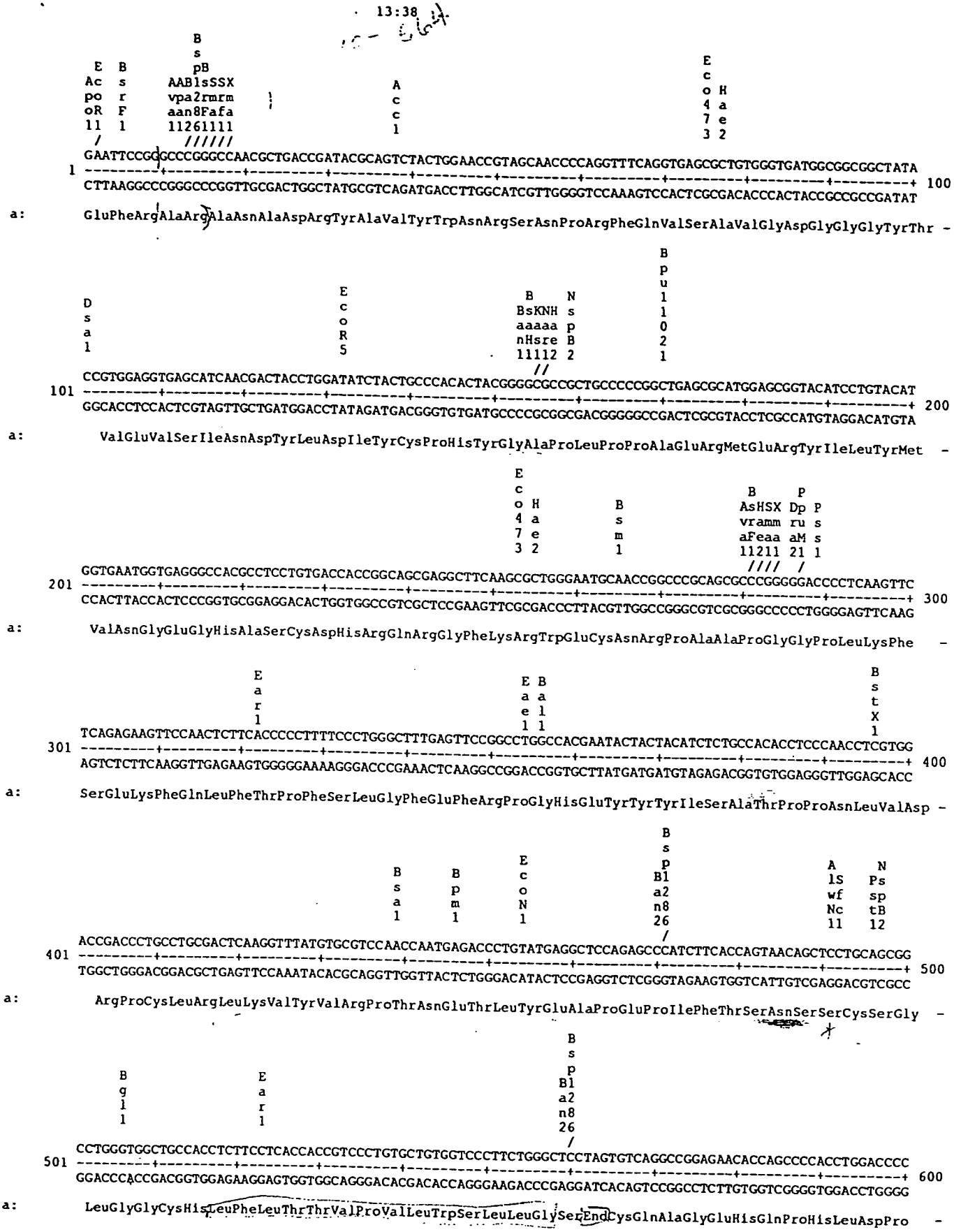
1kw A2 T7
Riboprobe
Template

To Page No.

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by	

Working File
Do Not Copy!

With 114 enzymes: *



```
                                  B
                     D   Es
                     s   ap
                     a   eM
                     1   11
    GTGACCTTTGCCCTCTGACCTGCCACGGCCACCTCCGAGACAAAATCCTTGCTGCTTCTCTTTCATGGTGCTGTCCCGCCGGAGGAGGCCATCCATCCGT
601 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 700
    CACTGGAAACGGGAGACTGGACGGTGCCGGTGGAGGCTCTGTTTTAGGAACGACGAAGAGAAAGTACCACGACAGGGCGGCCTCCTCCGGTAGGTAGGCA

a:  ValThrPheAlaLeuEndProAlaThrAlaThrSerGluThrLysSerLeuLeuLeuLeuPheHisGlyAlaValProProGluGluAlaIleHisProSer -

                                B
                  P             s
                  Dp P          u               B    S                                  BS
                  ru s          3               b    t                                  gf
                  aM s          6               s    y                                  li
                  21 1          1               1    1                                  11
                  /                                                                     /
    CCCTGGGATGCAACATGGGGTCCCAATGCCTGAGGAGAAGACCCCCCCCCAAGGCTGACTCGCTTTCACCAGGGCCACCAGGGCCATCCAGTGTTGYATA
701 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 800
    GGGACCCTACGTTGTACCCCAGGGTTACGGACTCCTCTTCTGGGGGGGGGTTCCGACTGAGCGAAAGTGGTCCCGGTGGTCCCGGTAGGTCACAACRTAT

a:   LeuGlyCysAsnMetGlySerGlnCysLeuArgArgArgProProProLysAlaAspSerLeuSerProGlyProProGlyProSerSerVal???End -

    ATTCTTT
801 ------- 807
    TAAGAAA

a:   PhePhe  -
```

Enzymes that do cut:

Acc1	AlwN1	Apo1	Apa1	Ava1	Bal1	Ban1	Ban2	Bbs1	Bgl1	Bpu1102I	Bpm1	Bsa1
BsaH1	Bsm1	Bsp1286	BspM1	BsrF1	BstX1	Bsu361	Dra2	Dsa1	Eae1	Ear1	Eco473	EcoN1
EcoR1	EcoR5	Hae2	Kas1	Nar1	NspB2	PpuM1	Pss1	Pst1	Sfc1	Sfi1	Sma1	Srf1
Sty1	Xma1											

Enzymes that do not cut:

Aat2	Afl2	Afl3	Age1	ApaL1	Asc1	Ase1	Asp718	Asu2	Avr2	BamH1	Bcg1	Bcl1
Bgl2	BsaA1	BsaB1	BsiE1	BsiW1	BspE1	BspH1	BssH2	Bst1107	BstE2	Cla1	Dra1	Dra3
Drd1	Eam1105	Eco571	Esp31	Fsp1	HgiA1	Hinc2	Hind3	Hpa1	Kpn1	Mlu1	Mun1	Nco1
Nde1	NgoM1	Nhe1	Not1	Nru1	Nsi1	NspH1	Pac1	PflM1	Pme1	Pml1	Pvu1	Pvu2
Rsr2	Sal1	Sca1	SgrA1	SnaB1	Spe1	Sph1	Sse8387	Ssp1	Sst1	Sst2	Stu1	Swa1
Tth31	Tth32	Xba1	Xcm1	Xho1	Xho2	Xma3	Xmn1					

```
GAP of: Mlerk6.Pep  check: 6430  from: 1  to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

 to: A2.Pep  check: 4723  from: 1  to: 238

TRANSLATE of: a2.seq check: 6473 from: 83 to: 796
generated symbols 1 to: 238.
HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL] . . .

 Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
 CompCheck: 1254

        Gap Weight:  3.000      Average Match:  0.540
     Length Weight:  0.100   Average Mismatch: -0.396

           Quality:  137.9             Length:    246
             Ratio:  0.741               Gaps:      6
Percent Similarity: 67.416   Percent Identity: 48.876

Mlerk6.Pep x A2.Pep                    16:30  ..

       1 .....................RARANADRYAVYWNRSNPRFQVSAVG  26
                               .: | ::|.|||||.||.::.
       1 MAAAPLLLLLLLVPVPLLPLLAQGPGGALGNRHAVYWNSSNQHLRR....  46

      27 DGGGYTVEVSINDYLDIYCPHY.......GAPLPPAERMERYILYMVNGE  69
           :||||:|.:||||||||||||       ||. .|::  |.|:||||. :
      47 ..EGYTVQVNVNDYLDIYCPHYNSSGVGPGAGPGPGGGAEQYVLYMVSRN  94

      70 GHASCDHRQRGFKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHE 119
         |. .|: .| |||||||||| || :|:|||||| :.:||||:||::|||
      95 GYRTCNASQ.GFKRWECNRPHAPHSPIKFSEKFQRYSAFSLGYEFHAGHE 143

     120 YYYISATPPNLVDRPCLRLKVYV......RPTNETLYEAPEPIFTSNSSC 163
         ||||| ||.: :.:.|||:||:|      :..:.:. . |: .:..| ..
     144 YYYIS.TPTHNLHWKCLRMKVFVCCASTSHSGEKPVPTLPQFTMGPNVKI 192

     164 SGLGGCHLFLTTVPVL.WSLLGS*..................... 186
         ..|:: .   . || | .|: |.
     193 NVLEDFEGENPQVPKLEKSISGTSPKREHLPLAVGIAFFLMTFLAS 238
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: C6.Pep check: 8194 from: 1 to: 201

TRANSLATE of: c6.seq check: 6086 from: 53 to: 655
generated symbols 1 to: 201.
HEKL 132-11, C6-no vector
2491,T7,DPC3266,DPC3267.DPC3274,DPC3275
SR1810 KOZLOSKY
file: [BERTLESJ.HEKL]C6.SEQ . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	118.5	Length:	216
Ratio:	0.637	Gaps:	7
Percent Similarity:	61.988	Percent Identity:	46.199

Mlerk6.Pep x C6.Pep 16:31 ..

```
   1 ..................RARANAD.RYAVYWNRSNPRFQVSAVGDGGGY  31
                        . |:... |..||||.||||:        |:
   1 MRLLPLLRTVLWAAFLGSPLRGGSSLRHVVYWNSSNPRLL......RGDA  44

  32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCD.HRQRG  80
     .||:::|||||| ||||::| ||.:. | : ||||: .|..||: . .|:
  45 VVELGLNDYLDIVCPHYEGPGPPEGP.ETFALYMVDWPGYESCQAEGPRA  93

  81 FKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNL 130
     :||| |   . | |.:.||||:| |||||||||| ||..|||||...|:
  94 YKRWVC...SLPFGHVQFSEKIQRFTPFSLGFEFLPGETYYYISVPTPES 140

 131 VDRPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCH.......... 170
      :. ||||.| | ..: .  .|...  .::|:.||: |..
 141 SGQ.CLRLQVSVCCKERKSESAHPVGSPGESGTSGWRGGDTPSPLCLLLL 189

 171 LFLTTVPVLWSLLGS* 186
     |:| .:.:|: |
 190 LLLLILRLLRIL.... 201
```

```
GAP of: Mlerk6.Pep  check: 6430  from: 1  to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

 to: Elkl.Pep  check: 1665  from: 1  to: 240

TRANSLATE of: tele7.seq check: 2210 from: 308 to: 1345
generated symbols 1 to: 346.
[hollingsworth.tele7] ELKL-E7.SEQ + ELKL-E7-3PRIME.SEQ;
req#1262
mGel 97 #2491+ #2492-/ mGel101 DPC2236+ DPC2239+/ mGel104 DPC2258+
DPC2257-/mGel105 DPC2261- /mGel107 DPC2271+ 2272- 2273- 2274+ . . .

 Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
 CompCheck: 1254

        Gap Weight:  3.000      Average Match:  0.540
     Length Weight:  0.100   Average Mismatch: -0.396

           Quality:   82.7             Length:    248
             Ratio:  0.445              Gaps:      6
Percent Similarity: 46.067   Percent Identity: 28.652

Mlerk6.Pep x Elkl.Pep                    16:46  ..

     1 RARANADR.........YAVYWNRSNPRFQVSAVG.........DGGGY 31
       .||:.. :           :|:::  .    .:.:|:          .| |.
     1 MARPGQRWLGKWLVAMVVWALCRLATPLAKNLEPVSWSSLNPKFLSGKGL 50

    32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQRGF 81
       .: ..|.| |||.||: :|. |     | | ||:|..|. |.|.    .
    51 VIYPKIGDKLDIICPRAEAGRP....YEYYKLYLVRPEQAAACSTVLDPN 96

    82 KRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNLV 131
          .|||   |:..::|. ||| |.|  :|:||:. |:||..|.. ..|
    97 VLVTCNR...PEQEIRFTIKFQEFSPNYMGLEFKKHHDYYITSTSNGSLE 143

   132 D........RPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCHLFL 173
       :          ..  :|:.::...:.   .||.: ||..| .: ....:
   144 GLENREGGVCRTRTMKIIMKVGQDPNAVTPEQLTTSRPSKEADNTVKM.A 192

   174 TTVPVLWSLLGS*..................................... 186
       | .|. :: ||.
   193 TQAPGSRGSLGDSDGKHETVNQEEKSGPGASGGSSGDPDGFFNSKVAL 240
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: Lerk5.Pep check: 8553 from: 1 to: 240

TRANSLATE of: lerk5.leg check: 889 from: 1 to: 1002
generated symbols 1 to: 334.
Coding region of human LERK-5.

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	83.2	Length:	250
Ratio:	0.447	Gaps:	5
Percent Similarity:	47.727	Percent Identity:	27.841

Mlerk6.Pep x Lerk5.Pep 16:59 ..

```
  1 ..................RARANADRY....AVYWNRSNPRFQVSAVGDG  28
                       .|.. .:     ::|||.||.:|      .|
  1 MAVRRDSVWKYCWGVLMVLCRTAISKSIVLEPIYWNSSNSKFL.....PG  45

 29 GGYTVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQ  78
    .|..: . |.| |||.||. :.  ...:. | | :|||: :. ..|. :.
 46 QGLVLYPQIGDKLDIICPKVDS..KTVGQYEYYKVYMVDKDQADRCTIKK  93

 79 RGFKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPP 128
      . . ::|   | |:..:||. ||| |.|  :|:||....:||.||.. .
 94 ENTPLLNC...AKPDQDIKFTIKFQEFSPNLWGLEFQKNKDYYIISTSNG 140

129 NLVD.....................................RPCLRLKVY 141
    .| :                                    || |_- .
141 SLEGLDNQEGGVCQTRAMKILMKVGQDASSAGSTRNKDPTRRPELEAGTN 190

142 VRPTNETLYEAPEPIFTSNSSCSGLGGCHLFLTTVPVLWSLLGS*..... 186
    .|..... :  |:|  ..:.:.:.| :| ::: ..|::: :: ::
191 GRSSTTSPFVKPNPGSSTDGNSAGHSGNNILGSEVALFAGIASGCIIFIV 240
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 18

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: B61.Pep check: 4381 from: 1 to: 205

TRANSLATE of: b61.seq check: 6304 from: 74 to: 688
generated symbols 1 to: 205.
LOCUS HUMB61 1480 bp ss-mRNA PRI
DEFINITION Human B61 mRNA, complete cds.
ACCESSION M57730 M37476
KEYWORDS intermediate-early response gene. . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	128.5	Length:	212
Ratio:	0.691	Gaps:	4
Percent Similarity:	59.218	Percent Identity:	45.251

Mlerk6.Pep x B61.Pep 16:29 ..

```
   1 ...........RARANADRYAVYWNRSNPRFQVSAVGDGGGYTVEVSIN  38
                . |.|||..|:||.|||:|.      .::||:.| :|
   1 MEFLWAPLLGLCCSLAAADRHTVFWNSSNPKFR......NEDYTIHVQLN  44

  39 DYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQRGFKRWECNR  88
     ||:||.||||:.. .:.. ||.||||:|: |:.. |:.. ::  ||:|||
  45 DYVDIICPHYEDHSVADAAMEQYILYLVEHEEYQLCQPQSKDQVRWQCNR  94

  89 PAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNLVDRPCLRL 138
     |.|. || |:||||| ||||.|| ||:.||.||||| . ..  || ||||
  95 PSAKHGPEKLSEKFQRFTPFTLGKEFKEGHSYYYISKPIHQHEDR-CLRL 143

 139 KVYVRP..........TNETLYEAPEPIFTSNSSCSGLGGCHLF.LTTV 176
     || |..          ..|. ..|.:| .    |.:  ::.:|| |. .
 144 KVTVSGKITHSPQAHVNPQEKRLAADDPEVRVLHSIGHSAAPRLFPLAWT 193

 177 PVLWSLLGS*.. 186
     .:|:.||
 194 VLLLPLLLLQTP 205
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: Mc6.Pep check: 7024 from: 1 to: 168

TRANSLATE of: mc6.seq check: 5844 from: 2 to: 505
generated symbols 1 to: 168.
Sequence of murine C6 (LERK-4) as derived from the genomic clone (3.5 kbp Sst1 fragment).

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	111.3	Length:	196
Ratio:	0.663	Gaps:	7
Percent Similarity:	65.190	Percent Identity:	45.570

Mlerk6.Pep x Mc6.Pep 16:31 ..

```
  1 RARANADRYAVYWNRSNPRFQVSAVGDGGGYTVEVSINDYLDIYCPHYGA 50
                      :...||      ||:::|||||:||||:.
  1 ................LLRGDAV.......VELGFNDYLDIFCPHYES 25

 51 PLPPAERMERYILYMVNGEG.HASCDHRQRGFKRWECNRPAAPGGPLKFS 99
    | ||.:. | : ||||: .| .|:... ..:|.||:|..| || :|::||
 26 PGPPEGP.ETFALYMVDWSGYEACTAEGANAFQRWNCSMPFAPFSPVRFS 74

100 EKFQLFTPFSLGFEFRPGHEYYYISATPPNLVDRPCLRLKVYVRPTN.ET 148
    ||:| :|||.||||| ||..|||||...|: .:| ||||.| | ..: :.
 75 EKIQRYTPFPLGFEFLPGETYYYISVPTPESPGR.CLRLQVSVCCKESGS 123

149 LYEAPEPI.FTSNSSCSGLGGCH.......LFLTTVPVLWSLLGS* 186
     .|.:.|:  .::|:.||: |.|       |:|  :|:|: | .
124 SHESAHPVGSPGESGTSGWRGGHAPSPLCLLLLLLLPILRLLRVL. 168
```

GAP of: Mlerk6.Seq check: 8999 from: 1 to: 797

WORKING FILE
DO NOT COPY!

to: A2.Seq check: 9214 from: 1 to: 987

HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL]

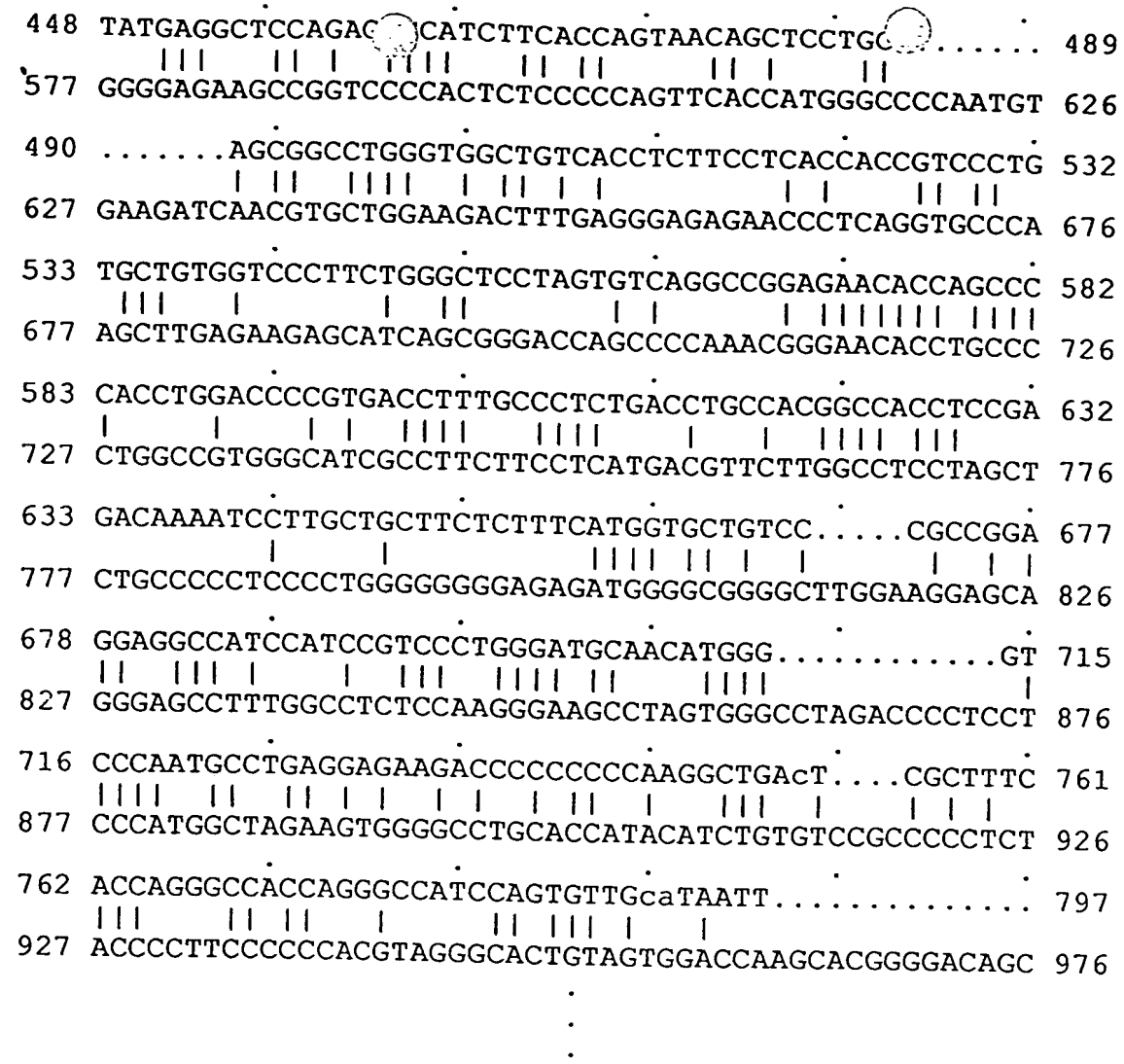
Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgapdna.Cmp
CompCheck: 6876

Gap Weight:	5.000	Average Match:	1.000
Length Weight:	0.300	Average Mismatch:	0.000
Quality:	362.8	Length:	1011
Ratio:	0.455	Gaps:	9
Percent Similarity:	56.016	Percent Identity:	56.016

Mlerk6.Seq x A2.Seq 16:33

```
   1 ..........................CGGGCCCGGGCCAACGCTGAC  21
 101 TGCCGCTGCTGCCGCTGCTGGCCCAAGGGCCCGGAGGGGCGCTGGGAAAC 150

  22 CGATACGCAGTCTACTGGAACCGTAGCAACCCCAGGTTTCAGGTGAGCGC  71
 151 CGGCATGCGGTGTACTGGAACAGCTCCAACCAGCACCTGCGG........ 192

  72 TGTGGGTGATGGCGGCGGCTATACCGTGGAGGTGAGCATCAACGACTACC 121
 193 ..........CGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATC 232

 122 TGGATATCTACTGCCCACACTA.....................CGGGGCG 150
 233 TGGATATTTACTGCCCGCACTACAACAGCTCGGGGGTGGGCCCCGGGGCG 282

 151 CCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTACATGGTGAA 200
 283 GGACCGGGGCCCGGAGGCGGGGCAGAGCAGTACGTGCTGTACATGGTGAG 332

 201 TGGTGAGGGCCACGCCTCCTGTGACCACCGGCAGCGAGGCTTCAAGCGCT 250
 333 CCGCAACGGCTACCGCACCTGCAACGCCAGCCAG...GGCTTCAAGCGCT 379

 251 GGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCAAGTTCTCAGAG 300
 380 GGGAGTGCAACCGGCCGCACGCCCCGCACAGCCCCATCAAGTTCTCGGAG 429

 301 AAGTTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGG 350
 430 AAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCACGCCGG 479

 351 CCACGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGGACCGAC 400
 480 CCACGAGTACTACTACATCTCCACGCCCACTCACAACC...TGCACTGGA 526
```

0046



448 TATGAGGCTCCAGAC CATCTTCACCAGTAACAGCTCCTGC 489
577 GGGGAGAAGCCGGTCCCCACTCTCCCCCAGTTCACCATGGGCCCCAATGT 626

490 .....AGCGGCCTGGGTGGCTGTCACCTCTTCCTCACCACCGTCCCTG 532
627 GAAGATCAACGTGCTGGAAGACTTTGAGGGAGAGAACCCTCAGGTGCCCA 676

533 TGCTGTGGTCCCTTCTGGGCTCCTAGTGTCAGGCCGGAGAACACCAGCCC 582
677 AGCTTGAGAAGAGCATCAGCGGGACCAGCCCCAAACGGGAACACCTGCCC 726

583 CACCTGGACCCCGTGACCTTTGCCCTCTGACCTGCCACGGCCACCTCCGA 632
727 CTGGCCGTGGGCATCGCCTTCTTCCTCATGACGTTCTTGGCCTCCTAGCT 776

633 GACAAAATCCTTGCTGCTTCTCTTTCATGGTGCTGTCC.....CGCCGGA 677
777 CTGCCCCCTCCCCTGGGGGGGGAGAGATGGGGCGGGGCTTGGAAGGAGCA 826

678 GGAGGCCATCCATCCGTCCCTGGGATGCAACATGGG.....GT 715
827 GGGAGCCTTTGGCCTCTCCAAGGGAAGCCTAGTGGGCCTAGACCCCTCCT 876

716 CCCAATGCCTGAGGAGAAGACCCCCCCCCAAGGCTGAcT....CGCTTTC 761
877 CCCATGGCTAGAAGTGGGGCCTGCACCATACATCTGTGTCCGCCCCCTCT 926

762 ACCAGGGCCACCAGGGCCATCCAGTGTTGcaTAATT..... 797
927 ACCCCTTCCCCCCACGTAGGGCACTGTAGTGGACCAAGCACGGGGACAGC 976

```
          Quality:  183.3          Length:    338
            Ratio:  0.554            Gaps:      3
Percent Similarity: 61.846  Percent Identity: 61.846

Mlerk6.Seq x Mc6.Seq                    14:13  ..

 99 TACCGTGGAGGTGAGCATCAACGACTACCTGGATATCTACTGCCCACACT 148
    |||||| || || ||||||| ||||| || |||| |||||||||| |
 20 .GTGGTGGAGCTGGGCTTCAACGATTACCTAGACATCTTCTGCCCACATT 68

149 ACGGGGCGCCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTAC 198
    | |    ||   |||||   | ||  ||  |||   |      | |||
 69 ATGAAAGCCCAGGGCCCC...CAGAAGGCCCGGAAACCTTTGCATTATAC 115

199 ATGGTGAATGGTGAGGGCCAC...GCCTCCTGTGACCACCGGCAGCGAGG 245
    |||||| |  |    ||| ||   |||| |   |   |  ||      |
116 ATGGTGGACTGGTCAGGCTACGAGGCCTGCACGGCAGAGGGGGCAAATGC 165

246 CTTCAAGCGCTGGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCA 295
    |||| |||||||| | |||     |||    || ||     | ||  |
166 CTTCCAGCGCTGGAATTGCTCGATGCCTTTTGCCCCTTTCAGCCCTGTTC 215

296 AGTTCTCAGAGAAGTTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAG 345
      |||||||| ||| | || | || ||| |||||  | ||||||||||||
216 GATTCTCAGAAAAGATTCAGCGCTACACACCCTTCCCGCTGGGCTTTGAG 265

346 TTCCGGCCTGGCCACGAATACTACTACATCTCTGCCACACCTCCCAACCT 395
    |||  ||||||  |    |||||||||||||| |   | ||||  |
266 TTCTTGCCTGGAGAGACTTACTACTACATCTCGGTGCCGACTCCGGAGAG 315

396 CGTGGACCGACCCTGCCTGCGACTCAAGGTTTATG... 430
      | |||    |||||| ||||| |||| | ||
316 TCCTGgCCG...GTGCCTGAGACTCCAGGTGTCTGTCT 350
$
```

Quality: 104.9 Length: 411
Ratio: 0.373 Gaps: 1
Percent Similarity: 39.858 Percent Identity: 39.858

Mlerk6.Seq x Lerk5.Seq 14:01 ..

```
 140 ............GCCCACACTACGGGGCGCCGCTGCCCCCGGCTGAGCG 176
                 |   |||    ||        ||||||    ||  |
1890 ATACCCACAGATAGGAGACAAATTGGATATTATTTGCCCCAAAGTGGACT 1939

 177 CATGGAGCGGTACATCCTGTACATGGTGAATGGTGAGGGCCACGCCTCCT 226
     |    | | |      | |  ||  |  |  ||           |
1940 CTAAAACTGTTGGCCAGTATGAATATTATAAAGTTTATATGGTTGATAAA 1989

 227 GTGACCACCGGCAGCGAGGCTTCAAGCGCTGGGAATGCAACCGGCC.... 272
     |      | | |||     ||   |     |  ||| |  |   ||
1990 GACCAAGCAGACAGATGCACTATTAAGAAGGAAAATACCCCTCTCCTCAA 2039

 273 ...CGCAGCGCCCGGGGGACCCCTCAAGTTCTCAGAGAAGTTCCAACTCT 319
        ||    || |    |    |||| ||| |    ||||| |||   |
2040 CTGTGCCAAACCAGACCAAGATATCAAATTCACCATCAAGTTTCAAGAAT 2089

 320 TCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGGCCACGAATACTAC 369
     ||| |||       |  |||  | || || | |     | | || || |||
2090 TCAGCCCTAACCTCTGGGGTCTAGAATTTCAGAAGAACAAAGATTATTAC 2139

 370 TACATCTCTGCCACACCTCCCAACCTCGTGGACCGACCCTGCCTGCGACT 419
      || ||| |  ||  |       | | || ||       || |
2140 ATTATATCTACATCAAATGGGTCTTTGGAGGGCCTGGATAACCAGGAGGG 2189

 420 C..................................................  420

2190 AGGGGTGTGCCAGACAAGAGCCATGAAGATCCTCATGAAAGTTGGACAAG 2239
```

HEK/ELK Meeting Minutes
B. Davison in the absence of Doug Cerretti.

Nicole Nelson summarized the recent screening of a murine embryonic cDNA library with a combination of LERKs(A2,C6) plus GSK beta kinase(Fred Fletcher's probe): Hybridization conditions were 42 C in 50% Stark's followed by washes at 63 C, 0.1X SSC.

13 initial positives were obtained of which 4 did not repeat; Nicole will rescreen these using a less stringent wash protocol. Currently, the sequence analysis indicates that the collection contains at least1 gsk Beta clone, 1 gsk Beta-like clone and interestingly, a new LERK, LERK-6.

All four cysteine residues are conserved while inspection of the carboxy terminal portion of the sequence indicates that LERK-6 fits into the GPI-linked class(similar to LERKS 1,3 and 4). The amino terminus of the protein is apparently lacking about 20 or 25 amino acid residues in the current clone. In the binding region, the LERK-6 DNA sequence displays about 70% identity with A2(LERK 3) corresponding to 288 bp of overlap.

Efforts are now being directed at identification of a source to obtain the human homologue.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Immunex Corporation
Attention: Stephen L. Malaska
Legal Affairs Department
51 University Street
Seattle, WA 98101

Deposited on Behalf of: Immunex Corporation (Docket No. 2826)

Identification Reference by Depositor:	ATCC Designation
Recombinant phage lambda gt10 vector, clone lambda 13M LERK-6 (murine)	75829

The deposit was accompanied by: __ a scientific description __ a proposed taxonomic description indicated above.

The deposit was received by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon Date:

Bobbie A. Brandon, Head, ATCC Patent Depository

Form BP4/9